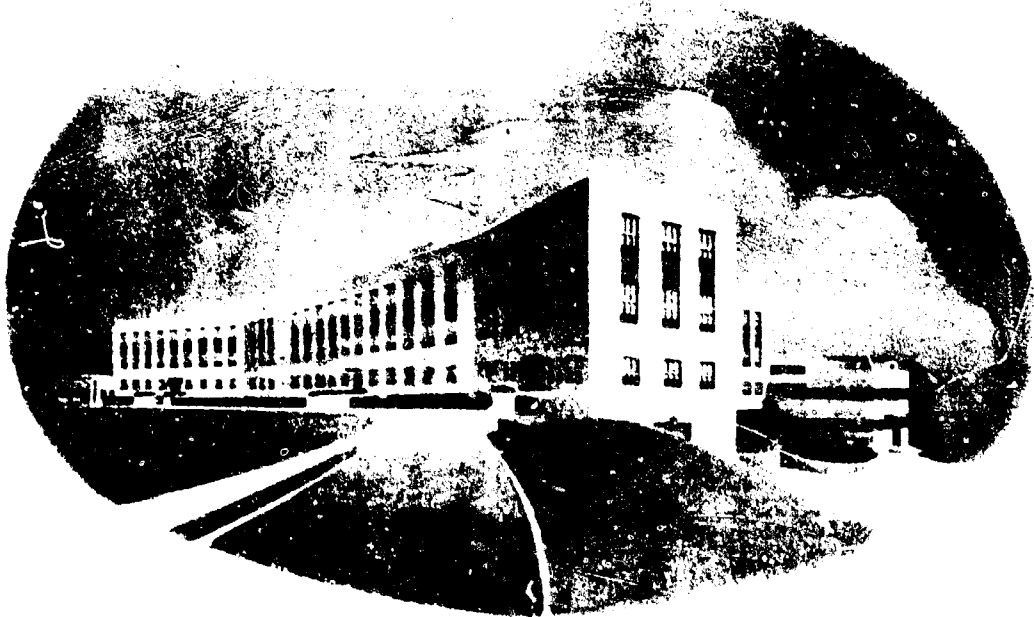


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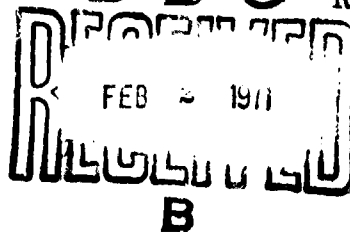
GROWTH OF ESCHERICHIA COLI IN HIGH PRESSURE HELIUM-OXYGEN GAS ATMOSPHERES

RESEARCH REPORT

MF12. 524. 014-9006AC9K

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Report No. 1



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**GROWTH OF ESCHERICHIA COLI IN HIGH PRESSURE
HELIUM-OXYGEN GAS ATMOSPHERES**

N. A. SCHLAMM
CDR MSC USN

J. E. PERRY
Microbiologist

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Report No. 1

**NAVAL MEDICAL RESEARCH INSTITUTE
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND 20014**

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ABSTRACT

Growth of Escherichia coli strain W was accelerated by pressurization to 68 atm (1000 psig) with helium-oxygen gas atmospheres. The acceleration appeared to be related to sequestration of iron and more efficient iron utilization by pressurized cells.

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A recent study by Schlamm, Turner, and Daily (Bacteriol. Proc. p. 99, 1970), which showed that alanine uptake in Staphylococcus aureus was increased by high atmospheric pressure, prompted us to investigate the effect of high pressure on bacterial growth. Some results of tests with Escherichia coli are described in this report.

For these studies, E. coli W (ATCC 9637) was grown in cotton-stoppered test tubes in 3.5 ml aliquots of Difco Minimal Broth Davis (MBD) made with triple distilled water. The aliquots were inoculated with cells from 15-hr cultures in MBD to a final concentration of 1 to 5×10^5 per ml. In a number of experiments the inoculum cells were washed three times in pH 7.0 phosphate buffer before use. Cell-free filtrate (CFF) of 20-hr cultures in MBD was prepared by centrifugation and filtration. Samples of 2,3-dihydroxybenzoylserine (DHBS) were isolated from E. coli supernatant fluid by Brot et al. (1). Pressurization of the cultures was accomplished by modification of a system described earlier (5). Cultures were placed in pressure vessels and flushed with 0.3% oxygen in helium to exclude room air. After pressurization to 68 atm (1000 psig) with flushing gas, the vessels were held at 37 C. At selected times, single vessels were depressurized and growth in cultures estimated optically at 600 nm with a Bausch and Lomb Spectronic 20 colorimeter. Parallel control cultures in 20% oxygen in helium atmospheres without pressurization [1 atm (0 psig)] were identically treated. Plating was done on representative cultures to test cell viability.

Pressurization of cultures inoculated with unwashed cells resulted in an acceleration in growth (Figure 1). The effect appeared to be only on the early phases of growth, probably on the lag phase; later growth phases and peak yield were unchanged. If measured as the time difference between 68 atm and 1 atm curves at an optical density value of 0.2, growth acceleration times in seven replicate experiments were observed to be about 1 hr (68 ± 10 min). Figure 1 also shows, by contrast, that pressurization did not accelerate growth in cultures inoculated with washed cells. These data suggest that an exogenous substance in the unwashed cell suspensions was carried over during inoculation and initiated early growth in the pressurized cultures.

Various microorganisms in iron-deficient basal media have been shown to synthesize growth promoting substances, chemically identified as iron chelators which are extractable from mature culture filtrates (2, 4, 7). To determine if our test organism also excreted such material, washed cells were grown without pressurization in MBD containing graded concentrations of CFF. Figure 2 shows that CFF indeed accelerated bacterial growth and that its effectiveness varied directly with concentrations over a range of 2 to 20 μ l/ml. No growth acceleration was elicited by concentrations below 2 μ l/ml. However, when growth was measured at 68 atm and with only 0.4 μ l/ml of CFF, an acceleration was observed (Figure 3). The mean of five replicate

tests in this case was 62 ± 4 min. The data thus indicate that CFF contains a substance which, in very low concentration, is effective as a growth initiator at 68 atm but not at 1 atm.

In final experiments, growth was measured in MBD containing, instead of CFF, low concentrations of DHBS, a growth promoting substance (3) characterized as a strong chelator secreted by *E. coli* in iron-deficient basal media (1, 6) and which is involved in active transport of iron (6). As shown in Figure 3, $1.0 \mu\text{M}$ DHBS had no effect on growth in a 1 atm culture. Figure 3 also shows, however, that DHBS in a 68 atm culture gave a growth acceleration time of about 70 min. The mean of five replicate tests was 75 ± 4 min. Higher concentrations of DHBS (to $50 \mu\text{M}$) generally increased growth rates, but acceleration times in pressurized cultures remained at about 75 minutes, regardless of DHBS concentration.

We conclude that a growth initiating substance secreted by *E. coli* W is rendered more effective by high pressure and that the effect may be related to sequestration of iron and more efficient iron utilization. Whether the data imply an increase in permeability to iron-bearing metabolites is not yet known. However, the previously cited studies of Schlamm et al. (Bacteriol. Proc. p. 99, 1970) lend strength to that implication.

We are indebted to Nathan Brot for his generous gift of 2,3-dihydroxybenzoyl-serine.

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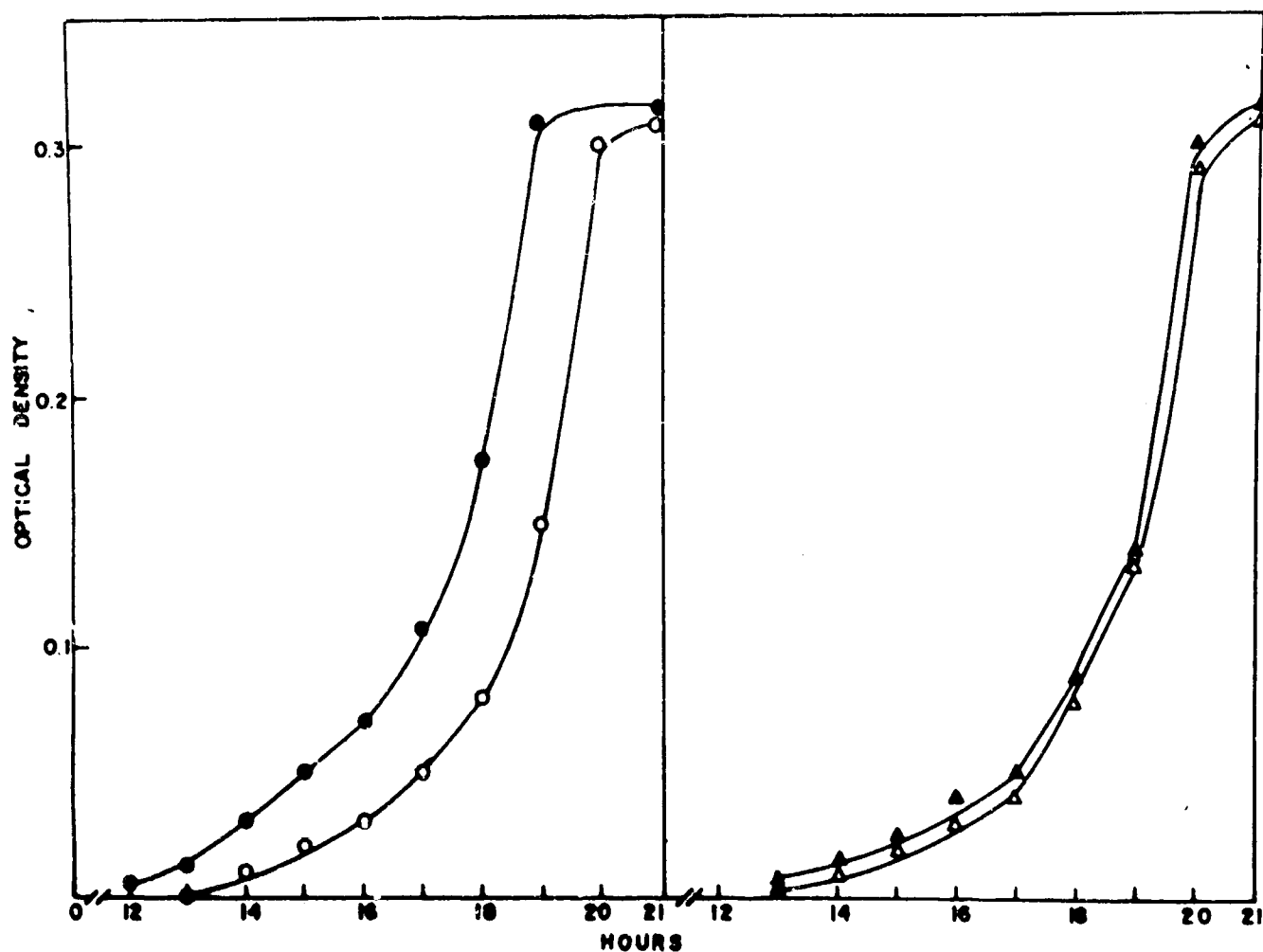


FIG. 1. Growth of *E. coli* W in Minimal Broth Davis inoculated either with washed or unwashed cells and in pressurized or nonpressurized oxygen-helium atmosphere. Symbols: ●, unwashed inoculum, 68 atm; ○, unwashed inoculum, 1 atm; ▲, washed inoculum, 68 atm; △, washed inoculum, 1 atm.

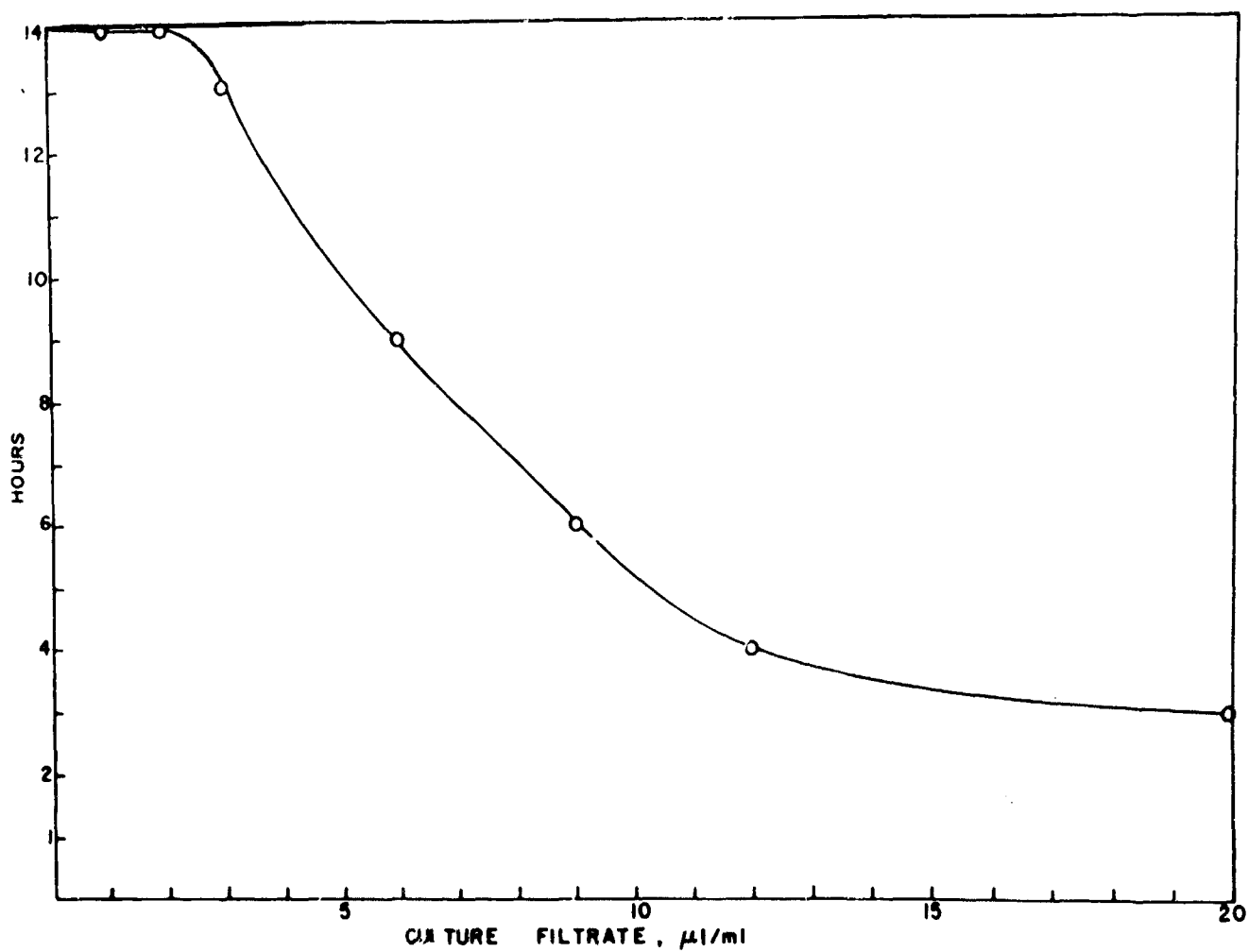


FIG. 2. Number of hours required for growth of *E. coli* W to reach a standard optical density value in Minimal Broth Davis supplemented with increasing concentrations of *E. coli* W mature culture filtrate.

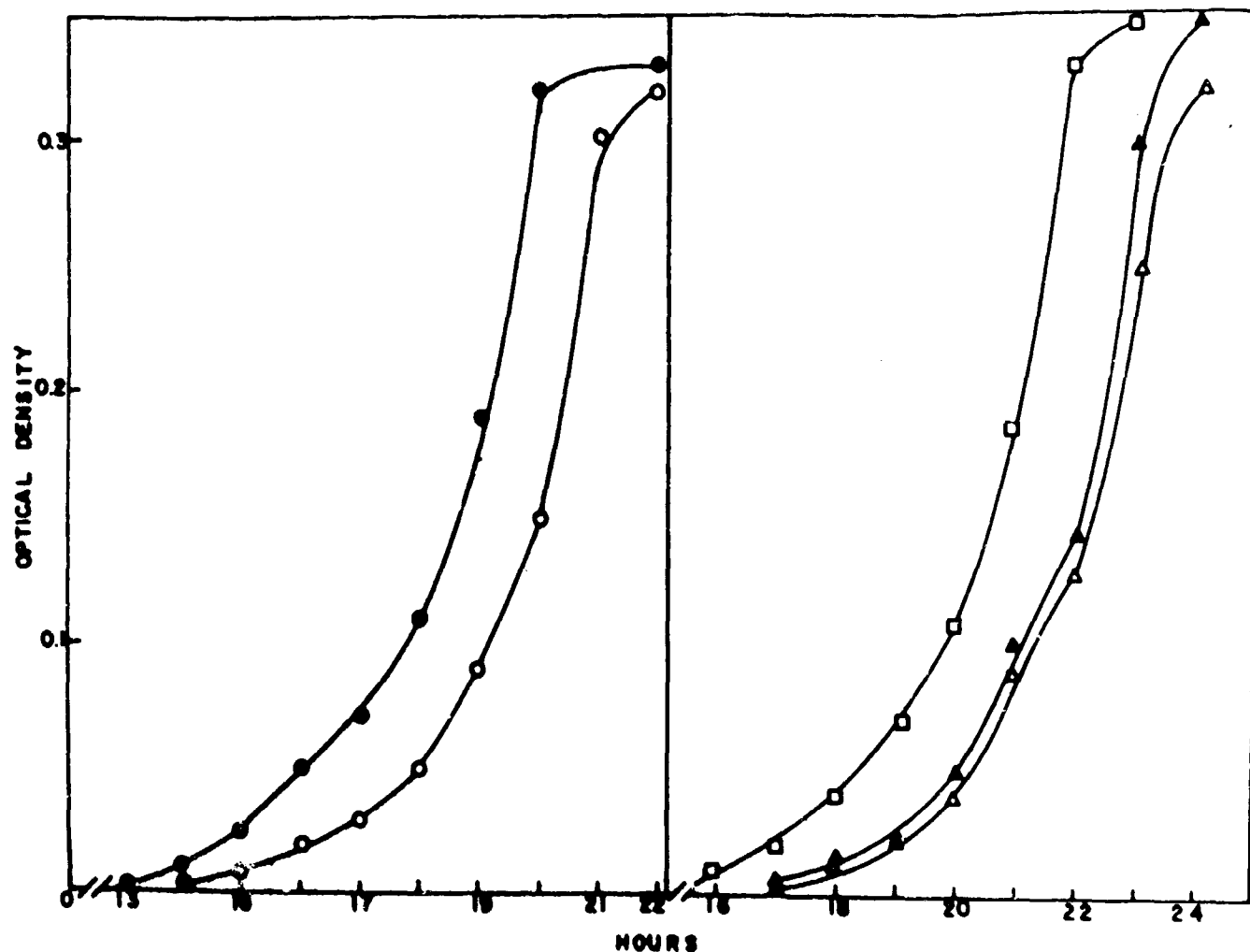


FIG. 3. Growth of *E. coli* W in Minimal Broth Davis containing either 0.4 μ l/ml of mature culture filtrate or 1.0 μ M of 2,3-dihydroxybenzoyl-serine (DHBS) and in pressurized or nonpressurized oxygen-hellum atmospheres. Symbols: ●, 0.4 μ l/ml filtrate, 68 atm; ○, 0.4 μ l/ml filtrate, 1 atm; □, 1.0 μ M DHBS, 68 atm; ▲, 1.0 μ M DHBS, 1 atm; △, no DHBS, 1 atm.

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